Departement für Kleintiere
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Patrick R. Kircher, PhD, Dipl. ECVDI

Arbeit unter wissenschaftlicher Betreuung von
Dr. med. vet. Nadja Sigrist, Dipl. ACVECC & ECVECC
PD Dr. med. vet. Annette PN Kutter, Dipl. ECVAA

Determination of reference intervals and comparison of venous blood gas parameters using standard and non-standard collection methods in 24 cats

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Karin Bachmann

Tierärztin

aus Bassersdorf, Zürich

genehmigt auf Antrag von

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Summary

The aim of this study was to determine in-house reference intervals for venous blood analysis with the RAPIDPoint 500 blood gas analyzer using blood gas syringes (BGS) and to determine whether immediate analysis of venous blood collected into lithium heparin (LH) tubes can replace anaerobic blood sampling into BGS.

Venous blood was collected from 24 healthy cats and directly transferred into a BGS and a LH tube. The BGS and LH tubes were compared using paired t-test or Wilcoxon matched-pairs signed-rank test, Bland-Altman and Passing-Bablok analysis. To assess clinical relevance, the bias or % bias between BGS and LH tubes was compared with the allowable total error (TEa) recommended for the respective parameter.

Based on the values obtained from the BGS, reference intervals were calculated for the evaluated parameters including blood gases, electrolytes, glucose and lactate. Values derived from LH tubes showed no significant difference for standard bicarbonate, whole blood base excess, hematocrit, total hemoglobin, sodium, potassium, chloride, glucose and lactate while pH, partial pressure of carbon dioxide and oxygen, actual bicarbonate, extracellular base excess, ionized calcium and anion gap were significantly ($p < 0.05$) different to the samples collected in BGS. Furthermore pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, ionized calcium and anion gap exceeded the recommended TEa.

Zusammenfassung

Ziel dieser Studie war es, unter Verwendung von standardisierten Blutgasspritzen (BGS), Referenzintervalle für venöse Blutgasanalysen auf dem RAPIDPoint 500 Blutgasanalysegerät zu bestimmen, und zu ermitteln, ob die sofortige Analyse von in Lithium Heparin (LH) Röhrchen gesammeltem venösen Blut die Verwendung von BGS ersetzen kann.
Von 24 gesunden Katzen wurde venöses Blut entnommen und direkt in BGS und LH Röhrchen transferiert. Die Resultate der BGS und LH Röhrchen wurden mit gepaartem t-Test oder Wilcoxon-Vorzeichen-Rang-Test, Bland-Altman und Passing-Bablok Analyse verglichen. Zur Beurteilung der klinischen Relevanz wurde der Fehler oder %Fehler zwischen BGS und LH Röhrchen mit dem erlaubten Gesamtfehler (TEa) des entsprechenden Parameters verglichen.
Basierend auf den Werten der BGS wurden Referenzintervalle für Blutgasparameter, Elektrolyte, Glukose und Laktat berechnet. Die Werte der LH Röhrchen zeigten keine signifikanten Unterschiede für Standardbikarbonat, Basenexzess im Vollblut, Hämatokrit, totales Hämoglobin, Natrium, Kalium, Chlorid, Glukose und Laktat, während pH, Kohlendioxid- und Sauerstoffpartialdruck, aktuelles Bikarbonat, Basenexzess im Extrazellulärraum, ionisiertes Kalzium und die Anionenlücke signifikant ($p < 0.05$) von den Werten in den BGS abwichen. Zudem überschritten pH, Kohlendioxid- und Sauerstoffpartialdruck, Basenexzess im Extrazellulärraum, ionisiertes Kalzium und die Anionenlücke den empfohlenen TEa.

Original Article

Journal of Feline Medicine and Surgery
1–10

DOI: 10.1177/1098612X16663269
jfms.com

This paper was handled and processed by the European Editorial Office (ISFM) for publication in JFMS

Determination of reference intervals and comparison of venous blood gas parameters using standard and non-standard collection methods in 24 cats

Karin Bachmann[1], Annette PN Kutter[2], Rahel Jud Schefer[1], Charlotte Marly-Voquer[2] and Nadja Sigrist[1]

Abstract

Objectives The aim of this study was to determine in-house reference intervals (RIs) for venous blood analysis with the RAPIDPoint 500 blood gas analyser using blood gas syringes (BGSs) and to determine whether immediate analysis of venous blood collected into lithium heparin (LH) tubes can replace anaerobic blood sampling into BGSs.

Methods Venous blood was collected from 24 healthy cats and directly transferred into a BGS and a LH tube. The BGS was immediately analysed on the RAPIDPoint 500 followed by the LH tube. The BGSs and LH tubes were compared using paired *t*-test or Wilcoxon matched-pairs signed-rank test, Bland–Altman and Passing–Bablok analysis. To assess clinical relevance, bias or percentage bias between BGSs and LH tubes was compared with the allowable total error (TEa) recommended for the respective parameter.

Results Based on the values obtained from the BGSs, RIs were calculated for the evaluated parameters, including blood gases, electrolytes, glucose and lactate. Values derived from LH tubes showed no significant difference for standard bicarbonate, whole blood base excess, haematocrit, total haemoglobin, sodium, potassium, chloride, glucose and lactate, while pH, partial pressure of carbon dioxide and oxygen, actual bicarbonate, extracellular base excess, ionised calcium and anion gap were significantly different to the samples collected in BGSs ($P<0.05$). Furthermore, pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, ionised calcium and anion gap exceeded the recommended TEa.

Conclusions and relevance Assessment of actual and standard bicarbonate, whole blood base excess, haematocrit, total haemoglobin, sodium, potassium, chloride, glucose and lactate can be made based on blood collected in LH tubes and analysed within 5 mins. For pH, partial pressure of carbon dioxide and oxygen, extracellular base excess, anion gap and ionised calcium the clinically relevant alterations have to be considered if analysed in LH tubes.

Accepted: 2 July 2016

Introduction

In recent years the use of blood gas analysers for rapid determination of oxygenation, ventilation, acid–base and electrolyte disorders in blood has become a standard in emergency rooms and critical care units of veterinary hospitals. The RAPIDPoint 500 (RP500; Siemens Healthcare) allows point-of-care assessment of blood gases, acid–base status, co-oxymetry, electrolytes, glucose and lactate within 60 s from a single whole blood sample.

While interpretation of oxygenation requires analysis of an arterial blood specimen, several studies in people concluded that measurements from venous blood accurately reflect the acid–base status of the patient.[1–3] In cats

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and dogs the venous pH is significantly lower compared with arterial pH, while venous partial pressure of carbon dioxide ($PvCO_2$) is significantly higher than arterial partial pressure of carbon dioxide, subsequently leading to significantly higher bicarbonate (HCO_3^-) concentrations but no significant changes in base excess (BE).[4,5] For the correct assessment of venous blood gases reverence intervals (RIs) for venous blood have to be determined.

Haematocrit, total haemoglobin, electrolytes, glucose and lactate are typically measured in venous blood. A venous sample is easier to obtain and facilitates evaluation of acid–base status, electrolyte disorders and glucose and lactate concentration in emergency situations.

Manufacturers of blood gas analysers recommend using specific blood gas syringes (BGSs) to obtain reliable results. The smallest syringes available have a volume of 1 ml. Cats are small and have a lower relative blood volume than dogs. Small amounts of blood withdrawal are therefore desirable. Measurement of venous blood gas parameters using multi-purpose lithium heparin (LH) tubes, which can subsequently be used for analysis of other blood parameters, decreases the amount of required blood. It has been stated that canine venous blood samples collected into LH tubes did not show significant changes in $PvCO_2$, pH, HCO_3^- or BE at eight different time points within 30 mins, with the exception of pH at 2 mins after sample collection compared with native blood analysed directly after collection.[6]

The aim of this study was to determine in-house RIs for venous blood analysis with the RP500 blood gas analyser using BGSs and to determine whether immediate analysis of venous blood collected into LH tubes can replace anaerobic blood sampling into BGSs in cats.

The null hypothesis was that venous blood samples collected in BGSs and their corresponding venous blood samples collected in LH tubes deliver the same results for the evaluated parameters when analysed within 5 mins on the same blood gas analyser.

Materials and methods

Animals and procedures

The study was approved by the Swiss federal ethics committee on animal research of the Canton of Zurich. Cats older than 1 year of age were recruited between April 2013 and January 2014. They were considered healthy based on history, physical examination, haematology and serum biochemistry (parameters specified in Table S1; see supplementary material). Informed owner consent was obtained for all procedures.

Blood was sampled from the jugular vein with the cats restrained in sternal recumbency. The head was flexed dorsally and the forelimbs extended downwards over the edge of a table. If cats did not tolerate this position without struggling they were positioned in lateral recumbency and blood was collected from the medial saphenous or the cephalic vein. The cats were not sedated. After disinfection of the skin, blood was aspirated into a 10 ml syringe (Omnifix; B Braun Medical AG) using a 22 G hypodermic cannula. Subsequently, the blood was filled into the following containers: 0.6 ml blood into a 1 ml BGS (BD Preset 1 ml, 30 IU calcium-balanced lithium heparin; Becton Dickinson), 1.3 ml blood into a LH tube (35 IU lithium heparin per ml blood; Sarstedt), 1.3 ml blood into a potassium–EDTA tube (Sarstedt) and 1 ml blood into a serum tube. Air bubbles in the BGS were immediately expelled and the syringe closed with a rubber cap. The LH tube was closed with a plastic screw cap. The BGS and corresponding LH tube were then analysed immediately with the RP500 (Siemens Healthcare; see analytical performance in the Supplementary material). This analyser uses ion-selective electrodes for analysis of pH, Na, K, iCa and Cl, modified potentiometry for PCO_2 (Severinghaus electrode) and amperometry for PO_2 (Clark electrode), glucose and lactate (enzyme electrodes). Quality control is performed three times daily using an automatic quality control cassette. Parameters not passing the analytical performance criteria (Table S2; see Supplementary material) are transiently deactivated. The BGS was analysed first, followed by the LH tube. BGSs were connected to the sample receipt of the analyser. For the LH tubes, the screw cap was opened immediately prior to analysis and 0.2 ml blood (amount required for the analysis) was aspirated into an uncoated 1 ml PVC syringe (Omnifix; B Braun Medical AG) and the syringe was then connected to the analyser. The potassium–EDTA tube and the serum tube were sent to the in-house laboratory for a haematology and serum biochemical profile. Sixteen different parameters provided by the blood gas analyser were analysed, including pH, $PvCO_2$, venous partial pressure of oxygen (PvO_2), actual bicarbonate (HCO_3^- act), standard bicarbonate (HCO_3^- std), whole blood BE (BE B), extracellular base excess (BE ecf), haematocrit (Hct), total haemoglobin (tHb), sodium (Na), potassium (K), ionised calcium (iCa), chloride (Cl), anion gap (AnGap), glucose and lactate.

Statistical analysis

Data were analysed with Microsoft Excel and two statistical software packages (GraphPad Prism 6 [GraphPad Software] and Analyse-it [Analyse-it Software]). A Shapiro–Wilk test was performed to confirm or reject normal distribution for every measured parameter. In-house RIs based on the immediately analysed samples collected in the BGSs were determined with the Reference Value Advisor add-in for Microsoft Excel.[7] In agreement with the American College of Veterinary Pathologists (ASVCP) guidelines for the determination of RIs in veterinary species,[8] a parametric method was used

(recommended for sample sizes of 20–40 with normal distribution). For parameters not showing normal distribution, values were Box-Cox transformed to achieve normality. Ninety percent confidence intervals were calculated for upper and lower limits, according to the guidelines.[8] Differences between results from BGSs and LH tubes were analysed using a paired *t*-test (confirmed normality) or Wilcoxon matched-pairs signed-rank test (rejected normality). Bland–Altman and Passing-Bablok analysis were used to evaluate the agreement between the two methods.[9,10] To assess clinical relevance, the bias was compared with the allowable total error (TEa) of the respective parameter defined by the ASVCP guidelines[11] For parameters not defined in these guidelines, TEa values defined for human medicine were used. For parameters where TEa is expressed as a percentage, percentage bias was calculated using BGSs as the gold standard (% bias = bias/mean of BGSs). The level of significance was set at $P < 0.05$.

Results

Animals

Twenty-five healthy cats were enrolled in the study. One cat had to be excluded as air had not been properly expelled from the BGS before capping. The age of the remaining 24 cats ranged from 1 to 15 years (median 4 years). Most cats (n = 18) were domestic shorthair; the other breeds represented were Maine Coon (n = 2), British Shorthair (n = 2), Persian (n = 1) and domestic longhair (n = 1). Twelve cats were male neutered, one was male entire and 11 were female spayed.

RIs were calculated from 24 cats. Two cats had to be excluded from the comparison between standard and non-standard collection method as the samples were analysed in the wrong order.

Twenty-two cats were sampled on the jugular vein and two cats on the medial saphenous and the cephalic vein.

RIs

Values for Na, iCa, Cl, glucose and lactate were not normally distributed, while the other nine parameters showed normal distribution. Tukey's test identified suspect data for Na, iCa and glucose (one sample each). These values are included in the RIs as they originated from different individuals, and no evidence for preanalytical error or inclusion of unhealthy animals was detected. Hct and tHb were only measured in nine individuals; therefore, no RI is reported. Values for iCa were available in 14 animals and only mean, median, SD, minimum and maximum are reported (Figure 1, Table 1).

Comparison of BGS and LH tubes

Values derived from LH tubes showed no significant difference for the parameters HCO_3^- std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate, while the parameters pH, $PvCO_2$, PvO_2, HCO_3^- act, BE ecf, iCa and AnGap were significantly different compared with the samples collected in BGSs (Table 2).

Time between the two measurements ranged between 2 and 5 mins (mean ± SD 2.7 ± 0.8 mins).

Analysis of the Bland–Altman plots revealed high bias and wide limits of agreement (LOA) for $PvCO_2$ and PvO_2. Smaller bias and narrower LOA were observed for pH, HCO3– act, BE ecf, iCa and AnGap. The nine parameters not showing a significant difference (HCO_3^- std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate) all had small bias and narrow LOA (Table 2, Figure 2).

Comparison of percentage bias with TEa as defined by the ASCVP guidelines showed that the parameters HCO_3^- act and HCO_3^- std, Na, K, Cl, glucose and lactate were within the proposed limits. All other parameters were compared with recommendations in people. Bias or percentage bias of pH, $PvCO_2$ and iCa exceeded human TEa. The parameters PvO_2 (7/22), AnGap (5/22) and BE ecf (2/22) showed samples exceeding human TEa. One of the samples exceeded TEa for BE B, but the *t*-test showed no significant difference between the BGS and LH tube for this parameter ($P = 0.5037$). The percentage bias of the two parameters Hct and tHb were within the recommendations for people (Table 3).

Passing–Bablok regression revealed proportional error for pH, $PvCO_2$, PvO_2, Na, iCa and AnGap (Figure 3).

Discussion

Critical illness in cats may lead to abnormalities in oxygenation/ventilation, acid–base haemostasis, Hct, electrolytes, glucose or lactate, among others. Blood gas analysers measuring these parameters allow immediate identification of potentially life-threatening abnormalities with a small blood sample. To our knowledge, this is the first study reporting feline venous blood gas RIs for the RP500 and showing that samples collected in multipurpose LH tubes allow clinically accurate determination for some of the parameters.

RIs depend on the specific method of measurement and need to be determined for each analyser. Studies on canine and feline acid–base values showed that acid–base values in healthy cats differ from values measured in healthy dogs,[4,12] illustrating the importance of using species-specific RIs to interpret blood gas results. Only a few studies have investigated feline venous blood gas RIs and they have been established on small numbers of five,[4] eight,[12] 10[13] and 13[14] animals, respectively. Our samples were obtained by venepuncture of conscious and restrained cats, while some of the previous studies on conscious cats used indwelling catheters for sampling.[4,13] Our results therefore include possible variations induced by stress as it might occur in a clinical situation. This led to a broader RIs than the studies in which blood was sampled from indwelling catheters. Apart from a

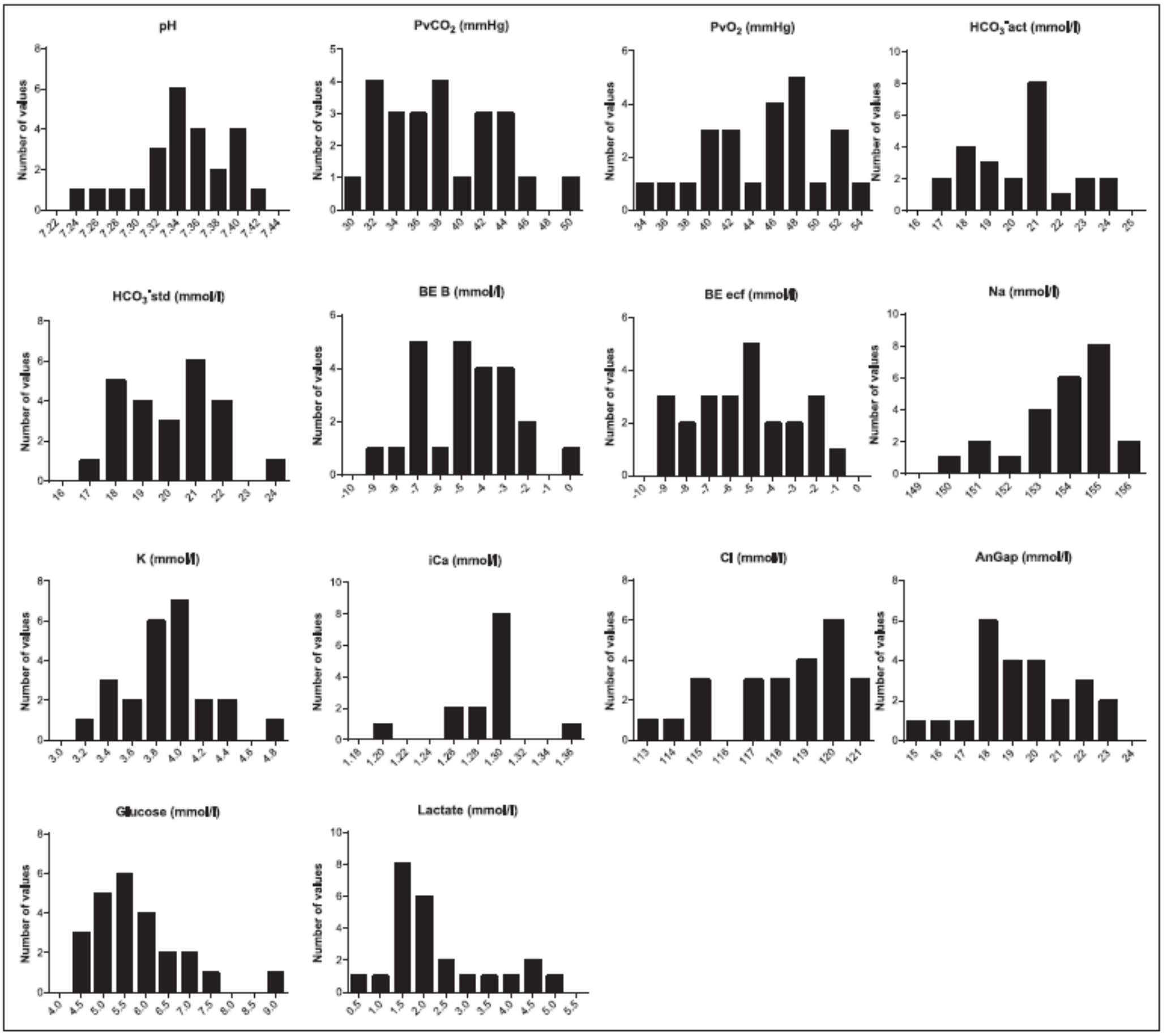

Figure 1 Histograms showing distribution of analysed parameters in 24 cats. $PvCO_2$ = venous partial pressure of CO_2; PvO_2 = venous partial pressure of oxygen; HCO_3^- act = actual bicarbonate; HCO_3^- std = standard bicarbonate; BE B = whole blood base excess; BE ecf = extracellular base excess; iCa = ionised calcium; AnGap = anion gap

considerably lower minimum level for $PvCO_2$ and a higher upper limit for pH, our RIs confirm the RIs reported by Middleton et al based on 13 cats.[14] The discrepancy in pH and $PvCO_2$ might be a result of hyperventilation and, consequently, respiratory alkalosis due to stress during venepuncture or represent a higher biological variation due to the larger sample size in our study. The RI for PvO_2 is of minor clinical relevance as oxygenation is generally assessed in arterial blood.

For lactate there is an ongoing debate about the width of the RI in this species and possible influences of factors like stress,[15,16] sampling technique, sample handling and sample population.[17] The most recent study published 2015 by Tynan et al on 47 cats determined a broader RI (0.67–5.44 mmol/l)[17] than previously suggested.[12,16] Our study supports the results of Tynan et al with a similar reference interval of 0.61–5.86 mmol/l for our 24 cats.[17] Further studies to assess possible factors leading to this high variation of lactate concentration in healthy cats are required.

A limitation of our study is the number of cats involved. Generally, RIs should be based on the largest number of samples possible. While in human medicine a minimum of 120 samples is required, the ASVCP guidelines for the determination of an RI in veterinary medicine advises against calculating an RI with a sample size <20 and recommends a sample size $\geqslant 40$ individuals.[8] Our sample size of 24 individuals is relatively small but allows reporting an RI with a reasonable validity. Hct and tHb were only available in nine cats as

Table 1 Reference intervals (RIs) of feline venous blood gas parameters

Parameter	n	Unit	Mean	Median	SD	Minimum	Maximum	RI	Lower 90% CI	Upper 90% CI
pH	24		7.344	7.343	0.048	7.230	7.423	7.244–7.444	7.218–7.272	7.415–4.472
$PvCO_2$	24	mmHg	38.2	37.6	5.2	30.7	49.2	27.3–49.1	24.5–30.3	45.9–52.1
PvO_2	24	mmHg	45.1	45.7	5.3	34.5	54.0	33.9–56.3	31.0–37.0	53.1–59.4
HCO_3^-act	24	mmol/l	20.3	20.6	2.1	16.9	24.4	15.9–24.7	14.7–17.1	23.4–25.9
HCO_3^-std	24	mmol/l	20.0	19.9	1.7	16.8	24.0	16.4–23.6	15.5–17.4	22.6–24.6
BE B	24	mmol/l	–4.9	–5.0	2.2	–9.2	–0.3	–9.5 to –0.3	–10.6 to –8.2	–1.6 to 1.0
BE ecf	24	mmol/l	–5.5	–5.5	2.4	–9.5	–0.7	–10.4 to –0.5	–11.7 to –9.1	–1.9 to 0.9
Na*	24	mmol/l	153.9	153.9	1.6	149.8	155.7	150.5–157.2	149.4–152.1	156.4–157.8
K	24	mmol/l	3.88	3.91	0.36	3.19	4.76	3.11–4.64	2.92–3.32	4.42–4.85
iCa*	14	mmol/l	1.29	1.30	0.04	1.19	1.35	–	–	–
Cl*	24	mmol/l	118	119	2	113	121	113–123	112–115	122–124
AnGap	24	mmol/l	19.3	19.2	2.1	15.4	23.4	14.8–23.8	13.7–16.1	22.5–25.0
Glucose*	24	mmol/l	5.8	5.6	1.1	4.4	9.0	4.3–8.8	4.1–4.6	7.5–10.7
Lactate*	24	mmol/l	2.32	1.85	1.24	0.50	5.23	0.61–5.86	0.41–0.89	4.39–7.50

*Parameters not showing normal distribution. For ionised calcium (iCa) no RI was determined and only mean, median, SD, and minimum and maximum values are reported
CI = confidence interval; $PvCO_2$ = venous partial pressure of CO_2; PvO_2 = venous partial pressure of oxygen; HCO_3^- act = actual bicarbonate; HCO_3^- std = standard bicarbonate; BE B = whole blood base excess; BE ecf = extracellular base excess; iCa = ionised calcium; AnGap = anion gap

Table 2 Comparison of feline blood gas parameters determined by blood gas syringes (BGSs) and lithium heparin (LH) tubes

Parameter	n	Unit	Mean BGS	Mean LH tube	Bias	95% CI	Limits of agreement		*P* value
pH*	22	–	7.343	7.389	0.046	0.038–0.054	0.010	0.082	<0.0001
$PvCO_2$*	22	mmHg	38.3	32.0	–6.3	–7.8 to –4.8	–13.0	0.4	<0.0001
PvO_2*	22	mmHg	44.7	49.7	5.0	2.8–7.1	–4.6	14.5	<0.0001
HCO_3^- act*	22	mmol/l	20.3	18.8	–1.5	–1.9 to –0.9	–3.7	0.8	<0.0001
HCO_3^- std	22	mmol/l	20.0	20.0	0	–0.1 to 0.3	–0.9	1.0	0.4871
BE B	22	mmol/l	–4.9	–5.0	–0.1	–0.4 to 0.2	–1.5	1.3	0.5037
BE ecf*	22	mmol/l	–5.5	–6.2	–0.7	–1.1 to –0.3	–2.6	1.2	0.0025
Hct	7	%	43.6	43.4	–0.2	–0.5 to 0.2	–0.9	0.6	0.3559
tHb	7	g/l	14.8	14.8	0	–0.1 to 0.1	–0.2	0.2	0.7358
Na	22	mmol/l	153.8	153.5	–0.3	–0.9 to 0.3	–2.9	2.3	0.2122
K	22	mmol/l	3.91	3.87	–0.03	–0.08 to 0.01	–0.21	0.14	0.0912
iCa*	13	mmol/l	1.29	1.22	–0.07	–0.10 to –0.03	–0.19	0.06	0.0015
Cl	22	mmol/l	118	118	0	–0.3 to 0.6	–1.9	2.2	0.6606
AnGap*	22	mmol/l	19.3	20.3	1.0	0.2–1.8	–2.6	4.5	0.0197
Glucose	22	mmol/l	5.8	5.8	0	–0.2 to 0.1	–0.7	0.6	0.5080
Lactate	22	mmol/l	2.4	2.5	0.1	–0.01 to 0.12	–0.2	0.3	0.0751

P values correspond to difference between BGS and LH tubes
*Parameters showing significant difference between BGS and LH tubes ($P < 0.05$)
CI = confidence interval of the bias; $PvCO_2$ = venous partial pressure of CO_2; PvO_2 = venous partial pressure of oxygen; HCO_3^- act = actual bicarbonate; HCO_3^- std = standard bicarbonate; BE B = whole blood base excess; BE ecf = extracellular base excess; iCa = ionised calcium; AnGap = anion gap; Hct = haematocrit; tHb = total haemoglobin

the spectrophotometric unit of the analyser was not available for some days of the study. These missing data also have an impact on HCO_3^- std and BE B as tHb is used for calculation of these parameters. If tHb is not available, 15 g/dl is used as a default value in the respective formulas. As all cats showed tHb within the RI (11.3–15.5 g/dl) in hematology performed in our in-house laboratory, this effect is negligible. A malfunction of the ion-selective electrode for iCa reduced the number of values for this parameter to 14 with no influence on other parameters. Our reported minimum and maximum for iCa, however, permits an estimate of the

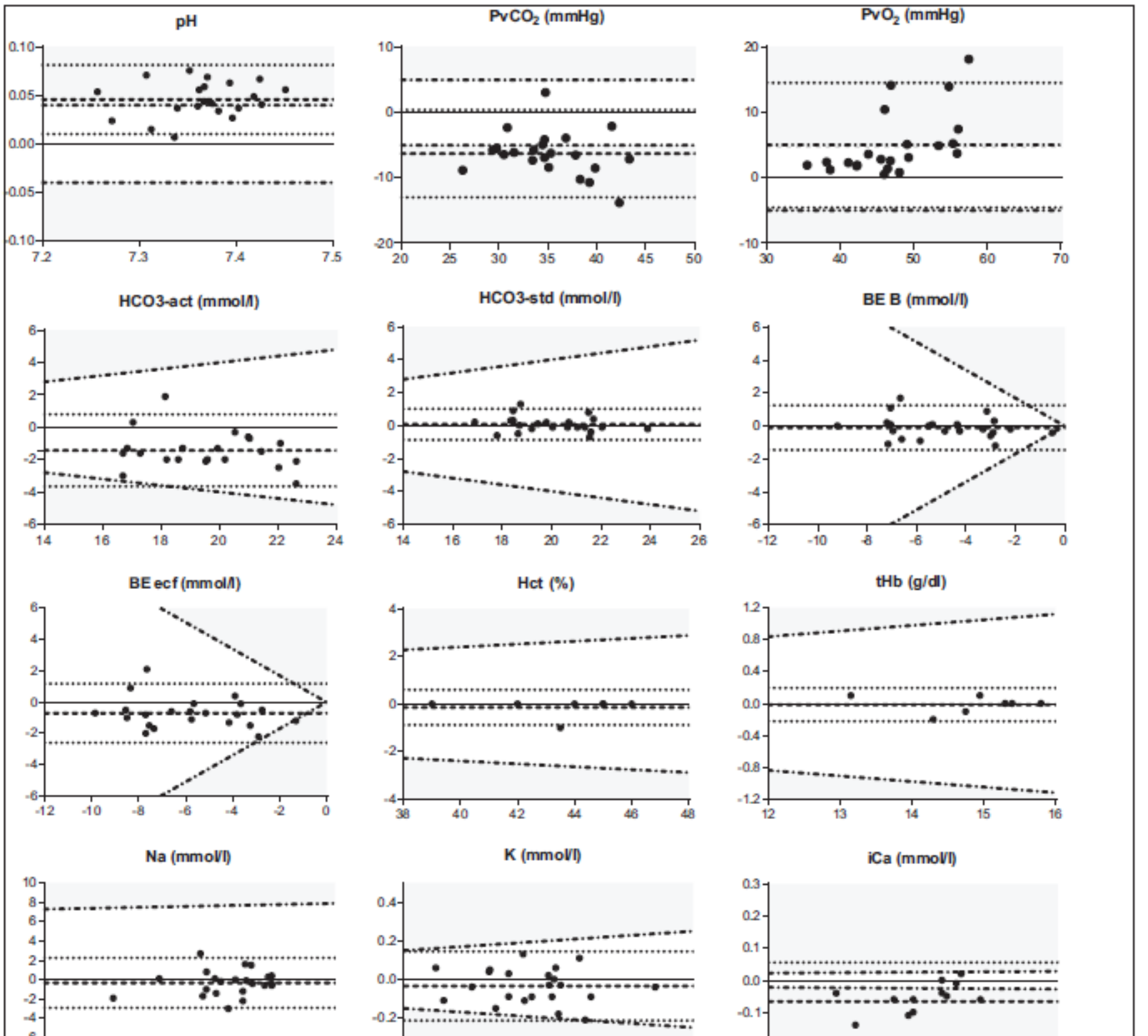

Figure 2 Bland–Altman plots comparing blood gas syringes and lithium heparin tubes. Grey shading indicates values exceeding allowable total error (TEa). $PvCO_2$ = venous partial pressure of CO_2; PvO_2 = venous partial pressure of oxygen; HCO_3^- act = actual bicarbonate; HCO_3^- std = standard bicarbonate; BE B = whole blood base excess; BE ecf = extracellular base excess; Hct = haematocrit; tHb = total haemoglobin; iCa = ionised calcium; AnGap = anion gap; LOA = limits of agreement

Table 3 Bias or percentage bias compared with recommended allowable total error (TEa)

Parameter	Bias/% bias	Range of bias	TEa	Source	Samples exceeding TEa
pH*	0.046	0.007–0.076	0.04	CLIA	14
$PvCO_2$*	−6.3 mmHg −16.4%	−13.8 to 3.1 mmHg −28.7 to 9.3%	5 mmHg or 8%	CLIA	16
PvO_2*	5.0 mmHg 11.2%	0.5–18.1 mmHg 1.1–37.4%	5 mmHg or 5%	RCPA	7
HCO_3^- act	−7.4%	−16.5 to 11.1%	20%	ASVCP	0
HCO_3^- std	0%	−3.3 to 7.2%	20%	ASVCP	0
BE B*	−2.0%	−133.3 to 22.7%	85%	BV	1
BE ecf*	12.7%	−171.4 to 24.1%	85%	BV	2
Hct	−0.5%	−2.3 to 0%	6%	CLIA	0
tHb	0%	−1.4 to 0.8%	7%	CLIA	0
Na	− 0.2 %	− 1.9 to 1.8%	5%	ASVCP	0
K	−0.9%	−4.8 to 3.5%	5%	ASVCP	0
iCa*	−5.1%	−16.0 to 1.6%	2%	BV	10
Cl	0%	−1.7 to 1.7%	5%	ASVCP	0
AnGap*	5.2%	−7.4 to 33.8%	11.3%	BV	5
Glucose	0%	−9.5 to 15.6%	20%	ASVCP	0
Lactate	4.2%	−9.8 to 28.0%	40%	ASVCP	0

*Parameters containing samples exceeding recommended TEa

$PvCO_2$ = venous partial pressure of CO_2; CLIA = clinical laboratory improvement amendments proficiency testing limits (1988); PvO_2 = venous partial pressure of oxygen; RCPA = Royal College of Pathologists of Australasia and the Australasian Clinical Biochemist Association quality assurance program; HCO_3^- act = actual bicarbonate; ASVCP = American Society for Veterinary Clinical Pathology; HCO_3^- std = standard bicarbonate; BE B = whole blood base excess; BV = Spanish Society of Clinical Chemistry and Molecular Pathology table of desirable quality specifications based on biological variation (update 2004); BE ecf = extracellular base excess; Hct = haematocrit; tHb = total haemoglobin; iCa = ionised calcium; AnGap = anion gap

physiological range of this parameter in healthy cats. Not differentiating between samples from different venous sites conflicts with the principle of maximum standardisation for the determination of RIs; however, only 2/24 samples were not aspirated from the jugular vein. Sampling from different venous sites reflects clinical practice, and for venous acid–base status[4] and lactate concentration[16] it has been demonstrated that results are comparable between different venous sites in cats.

Of the 16 parameters analysed in this study, HCO_3^- std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate showed no significant differences in measurements between sampling in BGSs and LH tubes. Consequently, medical conditions mainly influencing these parameters are reliably diagnosed with blood collected in LH tubes. The parameters pH, $PvCO_2$, PvO_2, HCO_3^- act, BE ecf, iCa and AnGap demonstrated significant changes in the LH tubes.

The main differences between the two sample containers used in this study are exposure to room air and the anticoagulant. While the BGSs are completely filled with blood up to the cone and air is expelled before capping, the LH tubes are designed for 1.3 ml blood but have a total volume of about 2 ml, leaving 0.7 ml of room air inside the tube after capping. These tubes have been chosen for the study as they are standardly used for collection of blood for analysis on our emergency biochemistry analyser during emergency service times. It has been shown previously in humans that air bubbles left in a syringe lead to a significant decrease in $PvCO_2$ within 3 mins and a significant rise in PvO_2 within only 2 mins.[18] Exposure to room air allows diffusion of an adequate amount of O_2 from room air with a higher PO_2 to blood and of CO_2 from blood with a higher PCO_2 to room air to change these two parameters significantly.

Our study shows a significant increase of PvO_2 and pH combined with a significant decrease of $PvCO_2$, HCO_3^- act and BE ecf in the tubes. The loss of CO_2 to room air in the tube leads to a significant decrease in $PvCO_2$ and subsequently to a significant increase in pH. Based on the formulae used for calculation of HCO_3^- act and BE ecf which contain pH and pCO_2, these two parameters are lower in the LH tubes. For the calculation of HCO_3^- std and BE B changes in pH and pCO_2 have less influence, therefore no significant difference was observed. As BE ecf is typically preferred over BE B in acid–base analysis,[19] this significant difference might be clinically relevant.

The study from 2004 by Richey et al,[6] comparing canine native venous blood analysed directly after collection with samples stored in the same LH tubes as in our study, did not find significant changes in $PvCO_2$, HCO_3^- and base excess on eight different time points within 30 mins. Two mins after sample collection pH

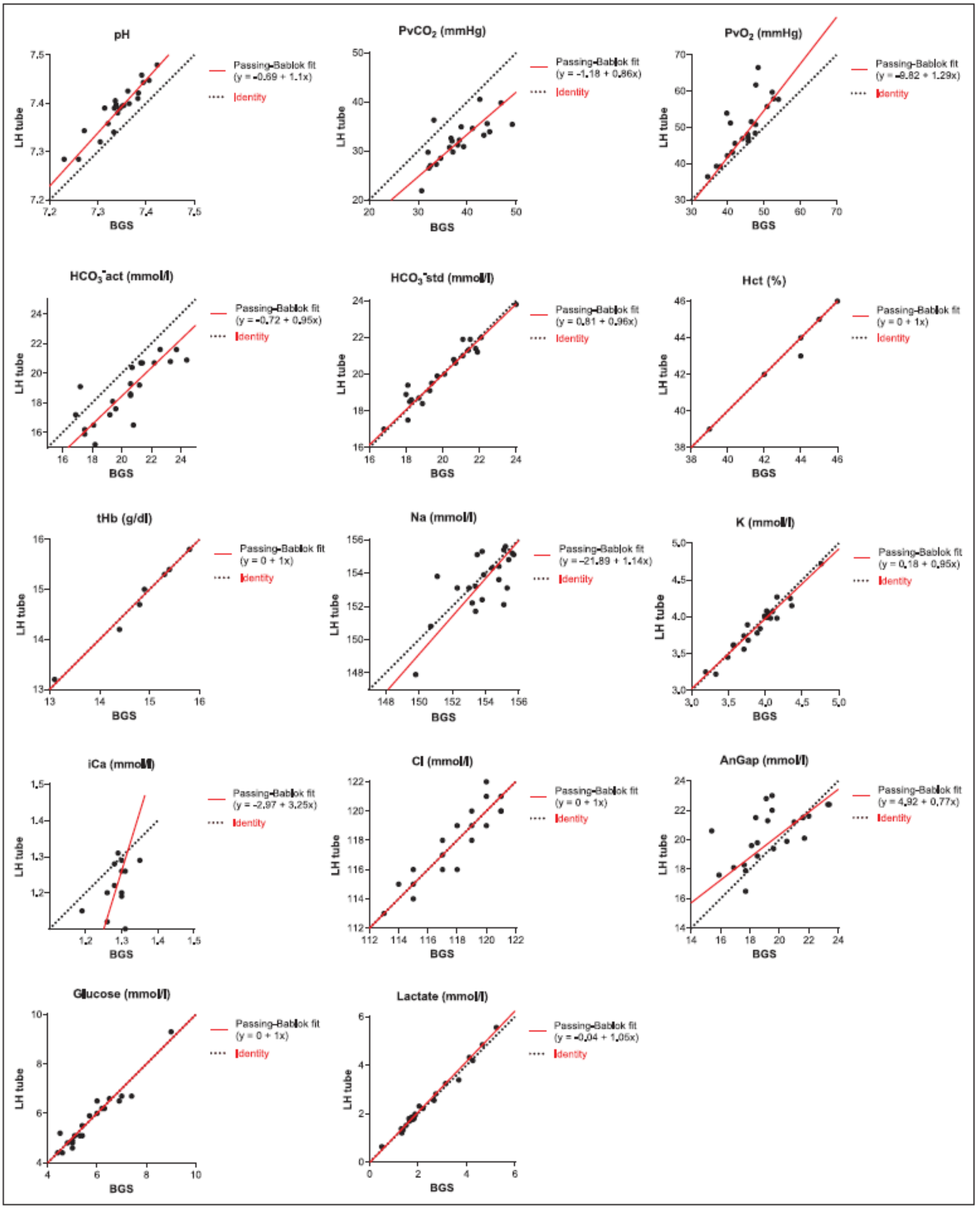

Figure 3 Passing–Bablok regression plots. BGS = blood gas syringe; LH = multi-purpose lithium heparin tube; $PvCO_2$ = venous partial pressure of CO_2; PvO_2 = venous partial pressure of oxygen; HCO_3^- act = actual bicarbonate; HCO_3^- std = standard bicarbonate; Hct = haematocrit; tHb = total haemoglobin; iCa = ionised calcium; AnGap = anion gap

was higher compared to native blood.[6] Comparing the two time points 2 mins and 5 mins after sample collection from that study with our samples, their samples showed the same tendencies, although did not reach significance except for the rise in pH after 2 mins.

The observed rise of the anion gap in our study is a further consequence of the decreased HCO_3^- act value. Anion gap is calculated using the formula

$$Na + K - \left(Cl + HCO_3^- act\right)$$

and therefore a lower HCO_3^- act value leads to a higher calculated anion gap.

The significant change in iCa between the samples collected in the BGS and the samples in the LH tubes is most likely based on the type of anticoagulant used. Heparin complexes calcium and thereby reduces the amount of measurable ionised calcium. Commercial BGS use dry calcium-balanced LH, which contains calcium to 'balance' the amount of iCa complexed by the heparin. Human studies support the use of calcium-balanced heparin for iCa analysis.[20,21] A rise in pH also reduces concentration of iCa in blood. The observed decrease of iCa (0.07 mmol/l) in the tubes exceeds the effect expected by the rise in pH of 0.04 according to a human study,[22] and was probably caused by the non-balanced heparin-complexing part of the iCa.

Statistical significance does not necessarily implicate clinical relevance. A difference in measured values of a certain parameter is only clinically relevant if it leads to a different decision regarding further treatment of the patient. The threshold for relevance varies greatly between different parameters, depending on how strictly physiologically regulated they are in vivo.[23] Considering this, the concept of TEa has been introduced in laboratory quality control. The specific TEa for a parameter of interest is derived from biological variation or clinical decision threshold and can vary between different species, analyte concentrations, clinical use and type of laboratory.[11] In human medicine, hundreds of parameters have been assessed and TEa for each parameter has been defined. For veterinary medicine the ASVCP TEa guidelines for biochemistry is, to date, the only source.[11] The parameters measured in our study, which are mentioned in these guidelines (Na, K, Cl, glucose lactate and HCO_3^-) all show a percentage bias lower than the recommended TEa, suggesting that the significant difference in HCO_3^- act may not be clinically relevant. As the bias for pH, $PvCO_2$ and iCa exceeded human recommendations and PvO_2 AnGap and BE ecf showed samples exceeding human TEa these differences presumably are clinically relevant in cats.

Using single instead of repeated testing may lead to over- or underestimation of the difference between the two methods for each sample pair but the calculated bias refers to the mean difference of all 24 samples and therefore provides an accurate assessment of the difference between the two containers. No internal validation to confirm the analytical performance stated by the manufacturer was performed for our RP500. However, the automated quality control performed three times daily confirmed accurate measurement within the predefined range.

Conclusions

Assessment of HCO_3^- act, HCO_3^- std, BE B, Hct, tHb, Na, K, Cl, glucose and lactate can be made based on blood collected in LH tubes. For pH, $PvCO_2$, PvO_2, BE ecf, AnGap and iCa the clinically relevant alterations have to be considered if analysed in LH tubes.

Conflicts of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding The authors received no financial support for the research, authorship, and/or publication of this article.

Supplementary material the following files are available:
Table S1: Blood parameters analysed as part of the inclusion criteria
Table S2: Analytical performance of the RAPIDPoint 500 blood gas analyser as stated by the manufacturer (Siemens)

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Danksagung

Mein Dank gilt meinen Betreuerinnen Frau Dr. med. vet. Nadja Sigrist und Frau PD Dr. med. vet. Annette Kutter für die Bereitstellung des Themas, die wissenschaftliche Betreuung sowie die große Unterstützung meiner Arbeit.

Herrn Prof. Dr. med. vet. Patrick Kircher danke ich für die Übernahme des Referats meiner Arbeit.

Ein herzliches Dankeschön auch an Frau Dr. med. vet. Rahel Jud für ihre Zusammenarbeit.

Abschließend möchte ich mich ganz besonders herzlich bei meiner Familie für ihre große und unermüdliche Unterstützung bedanken.

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